AN EXPERIMENTAL STUDY OF THE VISUAL PATHWAYS IN A SNAKE (*NATRIX NATRIX*)

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Remarkably few investigations have been made on the detailed structure of the ophidian central nervous system. Apart from observations by Warner (1931, 1947) on the forebrain and midbrain of *Crotalus*, only sporadic references occur in the literature to the morphology of particular cell masses and fibre systems in certain snakes. Papers by Gross (1903) on the optic chiasma, by Huber & Crosby (1933) on the optic tectum and by Frey (1937) on the basal optic root are of special interest with regard to the visual system. However, little has been added to our knowledge of central optic connexions in the snake since the early treatise by Bellonci (1888), on the optic system in vertebrates, which included observations on *Natrix*.

Histological studies of the ophidian brain have so far been based almost exclusively on normal material. Gross (1903) used the Marchi technique in *Natrix* and other reptiles following the removal of an eye, but his results were entirely negative. Recently (Armstrong, 1950) experiments were described in which silver impregnation was used to demonstrate axonal and terminal degeneration as a method for the detailed analysis of the primary visual pathways of *Lacerta vivipara*. The results were encouraging, and the same method is now used for a similar investigation in the grass snake, *Natrix natrix*.

The subject is of particular interest in view of the theory recently offered by Walls (1942) that snakes originated from ancestral burrowing lizards in which the eyes had almost disappeared. Certain peculiarities of the ophidian eye were interpreted as evidence that the visual system in modern snakes has been reconstructed. If this is correct, one might expect to find some significant differences between the central visual pathways of snakes and of other reptiles; it seemed likely that any such difference between *Natrix* and *Lacerta* would be made apparent by the present experiments.

MATERIAL AND METHODS

The animals studied were British specimens of *Natrix natrix*. They were kept in boxes maintained at a temperature of 25–30° C. Serial sections were prepared through the brains of seventeen snakes as follows:

(i) Two normal brains were fixed in a mixture of 90 c.c. of 50 % alcohol, 5 c.c. of formalin and 3·5 c.c. of glacial acetic acid. A transverse and a horizontal series of sections were cut in paraffin at 12 μ. The former was stained with gallocyanin, and the latter with cresyl violet.

(ii) The brains of fifteen snakes, in which the left optic nerve had been cut, were impregnated in block by Nonidez's (1939) silver method. Of these, nine were cut transversely, two sagittally and four in the horizontal plane, all at 7 μ. Post-operative survival times for the transverse series were 7, 8, 10, 13, 15, 25, 30, 88 and

136 days, for the sagittal series 13 and 22 days, and for the horizontal series 9, 11, 12 and 77 days.

All operations were performed under ether anaesthesia and with aseptic precautions. Care was taken to avoid traction at the optic chiasma. When the optic nerve had been cut a short length of it was removed, with the eye, in order to avoid the possibility of necrosis and infection. The orbital cavity was packed with penicillin-sulphathiazole powder. '*Oxycel*', a gauze type coagulant, was useful in arresting haemorrhage. The animals were ultimately killed by decapitation; the brain was exposed rapidly and fixed *in situ* for 24 hr. before removal.

NORMAL ANATOMY

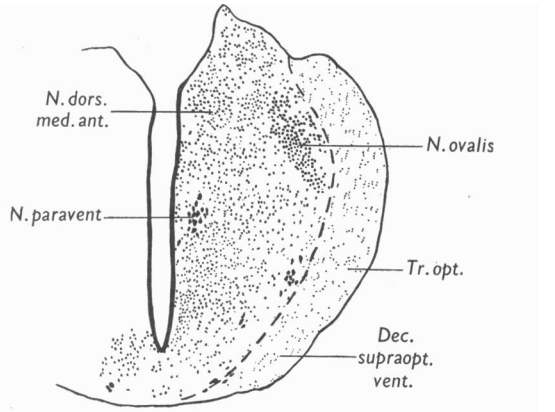
It is necessary to describe briefly the normal appearance of certain parts of the thalamus and midbrain related to the optic pathways. The terminology which is used includes many terms which have appeared frequently in the literature of reptilian neurology. It should be noted that some of these terms have not always been applied by different workers to corresponding structures. The need for caution in assuming homologies, even within the Reptilia, is therefore obvious.

Lateral geniculate nucleus. This nucleus lies in the lateral part of the diencephalon and is found at all levels except the extreme rostral pole. In sections through the middle and caudal parts of the thalamus it is clearly divisible into a lateral neuropil and a medial cell plate (Text-fig. 1*b*). The lateral neuropil is directly medial to the optic tract, and small cells are scattered amongst the fibres. A few larger rounded cells are present in a narrow zone immediately adjacent to the optic tract, where the neuropil is particularly dense. The lateral neuropil extends rostrally to a point just lateral to the caudal part of the nucleus ovalis (Text-fig. 1*a*). The medial cell plate is a broad band of mixed large and small cells. It is convex laterally but separated from the optic tract by the lateral neuropil. The large cells are fusiform or stellate; they send thick processes laterally to ramify within the lateral neuropil. When followed into the rostral part of the thalamus the medial cell plate becomes ill defined. It appears to separate into dorsal and ventral parts, both of which become indistinguishable from the nucleus dorsolateralis anterior. A strong fasciculus geniculatus descendens runs ventro-caudally from the lateral geniculate nucleus into the ventral part of the tegmentum.

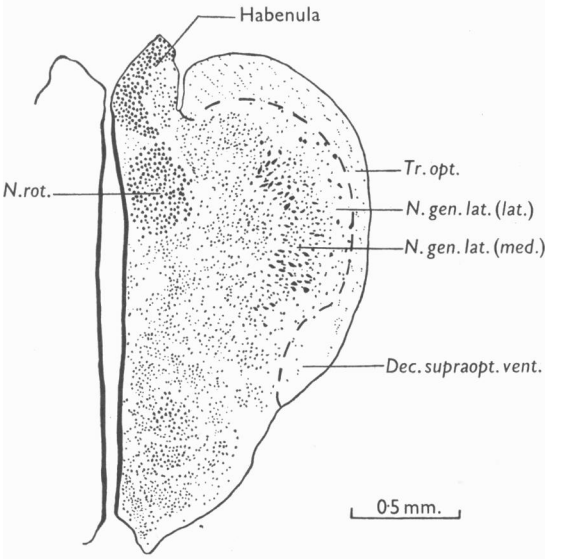
The lateral geniculate nucleus in *Natrix* is fundamentally similar to that of *Lacerta* (Beccari, 1923; Armstrong, 1950) and other reptiles. The main difference lies in the relatively poor differentiation of the medial cell plate in the snake. Illustrations by Warner (1931, 1947) suggest that in this respect the lateral geniculate nucleus in *Crotalus* resembles that of *Natrix*. It is to be noted, however, that Warner himself has followed Frederikse (1931) in applying the term 'corpus geniculatum laterale' only to that part which I have called the lateral neuropil.

Nucleus ovalis. This is a well-defined fusiform cell mass lying obliquely in the lateral part of the rostral pole of the thalamus (Text-fig. 1*a*). The component cells are small and densely packed. Caudally it is replaced by the medial cell plate of the lateral geniculate nucleus. There is no doubt that it corresponds to the nucleus ovalis described in other reptiles (Huber & Crosby, 1926; Cairney, 1926; Papez, 1935; Addens, 1938), and an almost identical formation in *Crotalus* was illustrated by

Warner (1931) in his fig. 12, but labelled as part of the lateral geniculate nucleus. A statement by Kappers (1947) that the nucleus ovalis is poorly developed or absent in snakes appears to be incorrect.



Text-fig. 1*a*.



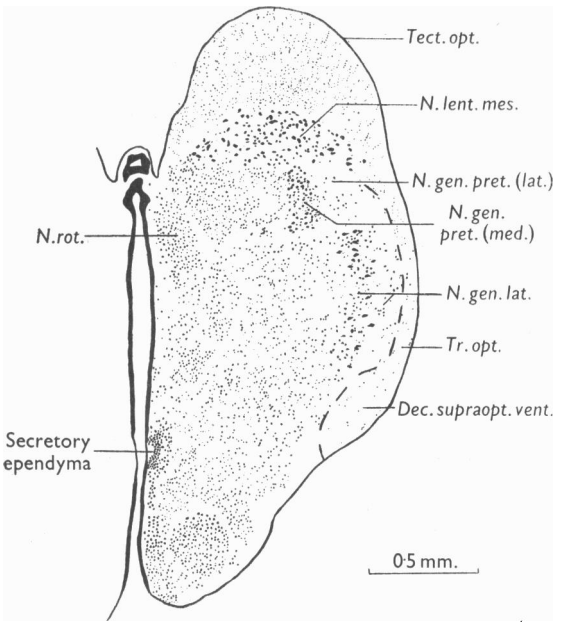
Text-fig. 1*b*.

Text-fig. 1. Scale drawings of transverse sections stained with gallocyanin: (*a*) through the rostral pole of the thalamus; (*b*) through the middle of the thalamus. For key to abbreviations see p. 288.

Pretectal region. The nucleus lentiformis mesencephali and the nucleus geniculatus pretectalis are readily identified (Text-fig. 2). They are similar in form and relations to the structures of the same name which were described in *Lacerta*. Sagittal silver series reveal a well-developed fasciculus geniculatus pretectalis descendens.

The nucleus pretectalis lies directly caudal to the nucleus lentiformis mesencephali, and is traversed by the usual fibre bundles passing between the diencephalon and the deeper layers of the optic tectum. Its caudal margin is well defined, but the boundary between it and the nucleus lentiformis mesencephali is much less distinct than in the lizard.

The nucleus posterodorsalis is a conspicuous feature in *Natrix*. It forms a small prominence medial to the optic tectum and overlying the posterior commissure (Pl. 3, fig. 9). Silver preparations show that it consists mainly of a characteristic neuropil, in which there are rounded areas composed of exceedingly fine fibres. Between these areas are a few small cells.



Text-fig. 2. Scale drawing of a transverse section through the pretectal region stained with gallocyanin. For key to abbreviations see p. 288.

A comparison of the pretectal morphology in different reptiles reveals a basic pattern, with variations between species only in the degree of differentiation of particular nuclei. The parts referred to here in *Natrix* certainly correspond to structures similarly named in *Alligator* (Huber & Crosby, 1926), *Sphenodon* (Cairney, 1926) and *Lacerta* (Beccari, 1923; Armstrong, 1950). Other workers such as Edinger (1899), de Lange (1913), Frederikse (1931), Warner (1931, 1947) and Kappers (1947) have failed to distinguish consistently between the nucleus lentiformis mesencephali, the nucleus geniculatus pretectalis and the nucleus pretectalis, and this is mainly responsible for prevailing confusion in the terminology of the reptilian pretectal region.

The nucleus posterodorsalis is of special interest in view of the considerable variation of its extent in different reptiles. It was first described by Huber & Crosby

(1926, 1933) in *Alligator* and *Anolis*, in which it appears to be moderately developed. In *Lacerta* it is extremely small. The so-called 'area pretectalis' of the Chelonia (Papez, 1935) is almost identical with the nucleus posterodorsalis of *Natrix*.

Optic tectum. Lamination of the tectum in *Natrix* is less elaborate than in *Lacerta*, but the same principal strata can be identified. The stratum zonale is a thin surface layer of neuropil containing few cells, and unlike that of the lizard is of uniform appearance over the whole extent of the tectum. The stratum opticum consists of the usual fibre bundles; but in *Natrix* they form a more sharply defined and uniform layer than in *Lacerta*. The most striking difference between the optic tecta of the snake and the lizard is in the stratum fibrosum et griseum superficiale. That of *Natrix* is relatively thinner, and in silver preparations does not present the division into substrata which is so obvious in the lizard. For further details of normal anatomy reference should be made to the comparative study of the reptilian optic tectum, including that of *Natrix*, by Huber & Crosby (1933).

EXPERIMENTAL RESULTS

Post-operative degeneration of the axons and their terminals was similar to that which occurs in *Lacerta*, although some differences were apparent, especially with regard to the time factor.

Axonal degeneration. Seven days after operation the severed retinal fibres had developed a slightly increased affinity for silver as compared with those from the normal side. After 8 days sporadic swellings were apparent in the degenerating fibres, and the difference in staining quality had become pronounced, making a striking contrast with the normal. In animals surviving between 9 and 12 days the degenerating fibres were identified without difficulty. Masses of fusiform swellings were arranged along them like beads, and in some places the slender segments between the swellings showed signs of rupture (Pl. 1, fig. 3). By 13 days axonal disintegration was advanced, and heavily staining fragments were present throughout the primary visual pathways (Pl. 1, fig. 4). These fragments had almost completely disappeared after 15 days, leaving only a small amount of argentophil debris. The 22- and 30-day series showed similar but rather more advanced stages of resorption. Traces of granular debris were still present after 77 and 88 days, but none at all could be seen in the 136-day specimen.

Terminal degeneration. Terminal degeneration of the bouton type (Hoff, 1932) occurred in various diencephalic and midbrain centres into which the degenerating retinal fibres could be traced. Typical terminal boutons could not be demonstrated in these areas in the normal state, but many fine argentophil ring-like structures were present 7 days after the operation. Between 8 and 12 days they were seen in increasing profusion and showed the intense affinity for silver which is a characteristic of degenerating terminals. The majority became enlarged and greatly thickened (Pl. 2, fig. 7), and in some cases solid terminal bulbs developed by total obliteration of the central lumen. It was clear, however, that all of the boutons did not undergo an identical sequence of changes. Many had attained a large size after only 8 days, others developed into solid bulbs without conspicuous enlargement, and

yet others remained both small and annular throughout. After 13 days the total number of rings and bulbs had diminished appreciably, and by 15 days all but a few had disappeared. Degeneration of the nerve terminals was associated with the usual swelling, vacuolation and ultimate fragmentation of the terminal arborizations of the retinal fibres. This resulted in a striking lack of neuropil in the primary visual centres from the 15th day onwards.

The material prepared in this investigation indicated a fairly constant time sequence for degeneration under the conditions in which the animals were kept, and only one specimen showed variation in this respect. This animal was killed 25 days after section of the optic nerve; it showed degenerative changes more typical of about 12 days.

The Visual Pathways

(i) *Optic nerve and chiasma.* The normal optic nerve in *Natrix* is divided by fine connective tissue septa into a large number of separate fascicles. The nerve fibres in each fascicle are disposed around a central column of cells which have large, vesicular nuclei and scant cytoplasm (Pl. 1, fig. 1); they are evidently glial elements, but their significance is uncertain. In longitudinal sections it seems that at least some of the cell columns are continuous from the eyeball to the optic chiasma. In the chiasma fasciculation of the optic nerve is lost and the cells become less numerous and segregated into short rows. The optic nerve as a whole divides into bundles, usually of very unequal size, which interlock with those from the opposite side. There is individual variation in the number, size and disposition of these bundles, but a frequent arrangement is for one nerve to form three bundles and the other nerve two. Section of the optic nerve resulted in degeneration of nearly all fibres in the central stump of the nerve. Decussation at the chiasma was almost complete, but a small proportion of the degenerating fibres remained uncrossed. The uncrossed element consisted of two groups of fibres, one of which passed on to the rostral surface of the homolateral optic tract, while the other turned sharply on to its ventral surface. The partial nature of the decussation was particularly obvious in the experiments of long duration, where the normal uncrossed component could easily be followed into the degenerated optic tract (Pl. 3, fig. 10).

After resorption of axonal debris, i.e. in the 77-, 88- and 136-day specimens, a few nerve fibres of normal appearance were still present in the central stump of the divided optic nerve (Pl. 3, fig. 11). The persisting fibres were scattered throughout the nerve, but were slightly more numerous dorsomedially than elsewhere.

Dorsal and ventral supraoptic (post-optic) decussations are closely related topographically to the optic chiasma. As the present experiments are not directly concerned with these systems it is sufficient to note that in *Natrix* they are essentially similar to those described in *Lacerta* (Armstrong, 1950).

(ii) *Optic tracts.* On emerging from the chiasma the optic tracts pass dorsolaterally on the surface of the hypothalamus. Section of the left optic nerve was followed, of course, by massive axonal necrosis in the contralateral tract. Degenerating fibres on the inner aspect of the tract were very intimately related to the adjacent hypothalamus. Some of them ran for short distances through the suprachiasmatic nucleus, and then rejoined the optic tract. Others, more laterally situated, entered the supraoptic

nucleus; here they intermingled in a plexiform manner with the outermost of the large neurosecretory cells which occur in this nucleus. However, it was clear on examination of serial sections that at least the majority of these fibres, and probably all of them, also passed back into the optic tract. No evidence of terminal degeneration could be found in the supraoptic nucleus, nor in any other part of the hypothalamus.

The basal optic root, or 'posterior accessory optic tract' is represented by coarse fibres which part from the optic tract as it approaches the level of the lateral forebrain bundle. They pass superficial to the pars ventralis of the ventral supraoptic decussation, and form a band-like tract which runs caudally to reach the nucleus opticus tegmenti. Division of the left optic nerve resulted in degeneration of the contralateral basal optic root. Degenerating terminals appeared in the nucleus opticus tegmenti in relation to the cell bodies and their dendrites (Pl. 2, fig. 6). Disappearance of the retinal fibres showed that the basal optic root is joined by a small number of fibres from the pars ventralis of the ventral supraoptic decussation. These, of course, did not degenerate. They are of two kinds: those which enter the nucleus opticus tegmenti with the retinal fibres, and others which sink into the lateral part of the hypothalamus and seem to constitute an interhypothalamic component of the ventral supraoptic decussation.

The main optic tract passes round the lateral forebrain bundle and runs dorsocaudally on the lateral surface of the thalamus. After section of the left optic nerve numerous degenerating fibres could be seen turning medially from the optic tract into the opposite lateral geniculate nucleus. These appeared to be both stem fibres and collaterals, but the distinction is by no means easy to make, even in serial sections. Profuse terminal degeneration occurred throughout the lateral neuropil (Pl. 2, fig. 5), degenerating boutons being specially numerous close to the optic tract. Fewer terminal rings and bulbs were encountered in direct relation to the cell bodies of the medial cell plate. This suggests that the synaptic relationship between the retinal fibres and the lateral geniculate nucleus is, as in *Lacerta*, predominantly axo-dendritic.

A few bundles of retinal fibres leave the optic tract dorsal to the lateral forebrain bundle, and pursue a separate course dorsocaudally through the lateral part of the thalamus. These represent the axillary optic tract of Huber & Crosby (1933). They are accompanied by fibres from the pars dorsalis of the ventral supraoptic decussation, from which they are indistinguishable in the normal state. Some of the more rostral axillary tract fibres pass through the nucleus ovalis, but no evidence of terminal degeneration could be found there. Many of these fibres terminate in the lateral neuropil of the lateral geniculate nucleus; others rejoin the main optic tract on its inner surface, but some continue independently into the lateral part of the pretectal region and the optic tectum.

A large number of crossed retinal fibres terminate in the pretectal region. Degenerating stem fibres and collaterals were traced medially from the main optic tract into the nucleus geniculatus pretectalis and the nucleus lentiformis mesencephali. These fibres form part of the so-called 'brachium tecti medialis' (Huber & Crosby, 1933). Degenerating terminals were very numerous throughout the lateral neuropil of the nucleus geniculatus pretectalis, and rather less so in the

nucleus lentiformis mesencephali. No evidence was found of retinal connexions with the nucleus pretectalis. Medial to the optic tectum a small and separate bundle of degenerating fibres could be traced from the optic tract into the nucleus posterodorsalis. This nucleus was also seen to be reached by a few degenerating fibres which passed through the nucleus lentiformis mesencephali in the brachium tecti medialis. The characteristic neuropil of the nucleus posterodorsalis was observed to fragment and finally to disappear almost completely after division of the opposite optic nerve (Pl. 3, fig. 9).

In *Natrix*, as in the lizard, the majority of the crossed retinal fibres ultimately enter the stratum opticum of the optic tectum. Only a small number of fibres persist in this layer of the tectum when the opposite optic nerve has been cut (Pl. 3, fig. 9). Small bundles of degenerating fibres from the main and axillary optic tracts could also be followed directly into the stratum fibrosum et griseum superficiale. From the stratum opticum many of the fibres were traced into the stratum zonale, but the majority turned abruptly from the stratum opticum into the underlying stratum fibrosum et griseum superficiale.

Terminal degeneration was extensive throughout the stratum fibrosum et griseum superficiale (Pl. 2, fig. 7), but was also seen over the whole surface of the tectum in the stratum zonale (Pl. 2, fig. 8). No evidence was found for the termination of retinal fibres in deeper layers; nor were any degenerating fibres seen to cross the midline in the tectum.

(iii) *Uncrossed retinal fibres.* The position of the uncrossed fibres at the chiasma has already been described. Their subsequent course was revealed equally well by two alternative methods of study; either by tracing axonal degeneration in the homolateral optic tract, or by following the normal uncrossed fibres in the contralateral tract after resorption of the crossed retinal fibres.

It was found that the uncrossed fibres at first remain superficial, on the rostral and ventral surfaces of the optic tract. Soon they swing over to the inner aspect of the tract, and converge to form a small, compact bundle. This could be followed round the lateral forebrain bundle into the neuropil which forms the rostral extremity of the lateral geniculate nucleus. A small amount of terminal degeneration was consistently located in this part of the homolateral geniculate nucleus.

Uncrossed fibres could not be traced into any other part of the lateral geniculate nucleus, nor to any of the other visual centres.

(iv) *Non-retinal components of the optic tract.* After degeneration and resorption of the crossed retinal fibres there remained a considerable number of normal fibres in the optic tract, quite apart from the uncrossed retinal component. Reference has already been made to the small proportion of optic nerve fibres which were unaffected by the operation. In the long duration experimental series these persisting fibres were traced without difficulty through the optic chiasma into the contralateral optic tract. A few of them, in the deeper part of the tract, appeared to enter the suprachiasmatic and supraoptic nuclei of the hypothalamus and could be traced no further; the majority, however, continued round the lateral forebrain bundle in the optic tract. Beyond this point it was impossible to distinguish them among the numerous non-retinal fibres which enter the optic tract at thalamic and pretectal levels.

The remaining non-retinal components may, for descriptive purposes, be divided into two groups, both of which join the optic tract on the lateral surface of the thalamus and in the pretectal region. These experiments do not, of course, indicate either the origin or the termination of these fibres. First, there is a contribution from the pars dorsalis of the ventral supraoptic decussation. Bundles of these fibres sweep into the lateral part of the thalamus in company with the axillary optic tract. Some of them incline laterally, and enter the main optic tract on its inner aspect. The second group consists of a large number of fibres which enter the optic tract from the lateral geniculate nucleus, the nucleus geniculatus pretectalis and the nucleus lentiformis mesencephali. They turn sharply dorsalwards and pass with the supraoptic fibres into the stratum opticum and the stratum fibrosum et griseum superficiale of the optic tectum.

DISCUSSION

Existing knowledge of the characteristics of axonal and terminal degeneration in the central nervous system of submammalian vertebrates is very limited. Experiments on the visual pathways of *Lacerta vivipara* revealed a succession of degenerative changes morphologically similar to those which are now well known in mammals, but they occurred much more slowly. It was observed, too, that the rate of degeneration in the poikilothermic lizard is controlled by the temperature of the environment. The present work on *Natrix* has now shown that the degenerative process in the snake is in certain respects different both from that of mammals and from that described in the lizard. In snakes kept at 25–30° C. the onset of visible degeneration in the severed axons and at the nerve endings is about as slow as in the lizard. Subsequent disintegration and resorption, however, are considerably more rapid; thus, more axonal debris remained in the optic nerve of *Lacerta* 11 weeks after the operation than was found in *Natrix* after only 22 days. Another feature of axonal degeneration in *Natrix* was the abundance of fusiform, bead-like swellings which developed between 9 and 12 days after the operation. Swellings are, of course, a common feature of axonal degeneration in mammals, and they were also seen in the lizard; but the regular occurrence of a stage when they predominate, almost to the exclusion of other forms of axonal distortion, appears to be unusual.

On the basis of these observations in reptiles it seems probable that appreciable variation in the characteristics of degeneration in different species, and possibly in different parts of the brain in the same species, may be encountered in future experimental studies on cold-blooded vertebrates.

With the aid of experimental data it is now possible to compare certain aspects of the organization of the visual system in *Natrix* and *Lacerta*. Attention has been drawn to the remarkable fasciculation of the optic nerve in the snake, and to the long rows of cells which it contains. In these features, however, it does not seem to differ fundamentally from the optic nerve of *Lacerta* or from those of other reptiles (Gross, 1903); the difference is only one of degree. The optic chiasma in *Natrix* contains fewer and, for the most part, larger decussating bundles than in *Lacerta*. This is in keeping with the very wide range of chiasma morphology which was shown by Gross to be a peculiarity of the Reptilia.

Perhaps the most striking feature of the present experimental results is the fact that they demonstrate such a close resemblance between the central optic pathways and the primary visual centres of the snake and those of the lizard. The crossed retinal fibres have an almost identical course and terminal distribution in the two animals. The only exception to this is the presence in *Natrix* of optic connexions with a well-differentiated nucleus posterodorsalis in the pretectal region. No such connexion could be found in *Lacerta*, where the nucleus posterodorsalis was found to be very poorly developed. This does not constitute an essential difference between snakes and lizards, for Huber & Crosby (1933) described a connexion between the optic tract and a fairly well-developed nucleus posterodorsalis in the American lizard, *Anolis*. The fascicle concerned was designated 'S' by Huber & Crosby, and assumed to be of retinal origin.

In both *Natrix* and *Lacerta* the experimental method has failed to demonstrate a direct retino-hypothalamic pathway, and there is certainly nothing comparable with the massive hypothalamic optic root stated by Frey (1937, 1938) to be present in certain mammals and amphibians. Other workers (e.g. Jefferson, 1940; Herrick, 1948) have already questioned the validity of Frey's interpretation. Similarly, no evidence has been found in the reptiles studied that any retinal fibres terminate in the nucleus ovalis. Certain axillary optic tract fibres were seen to pass through this nucleus; it therefore seems possible that optic fibres described in normal preparations of this region (e.g. by Papez, 1935, in turtles) may also have been fibres of passage. Cairney (1926), Papez (1935) and Addens (1938) believe that the reptilian nucleus ovalis may be homologous with the nucleus of Bellonci in amphibians. This hypothesis is called into question by the absence of optic connexions with the nucleus ovalis. On the other hand, it still remains to be proved experimentally that such a connexion does in fact exist with the amphibian nucleus of Bellonci as is commonly believed.

The uncrossed optic pathway in *Natrix* is very similar to that of *Lacerta*, but it was possible to trace these fibres with rather more ease and precision in the present experiments than in those on the lizard. Some evidence was obtained in *Lacerta* that uncrossed fibres may terminate in the lateral neuropil at the rostral extremity of the lateral geniculate nucleus; it is now clear that most and perhaps all of the uncrossed fibres in *Natrix* terminate in a corresponding area of the geniculate nucleus. In both snake and lizard this part of the lateral geniculate nucleus also receives crossed retinal fibres. It is just possible that a few of the uncrossed fibres continue dorsocaudally in the optic tract, becoming obscured in the available material by the numerous non-retinal fibres which enter the tract as it runs over the surface of the thalamus. It should be noted, however, that no terminal degeneration could be found in the homolateral pretectal region or optic tectum of either *Natrix* or *Lacerta*.

An interesting finding in *Natrix* is the presence of a number of fibres in the optic nerve which showed no sign of degeneration as much as 19 weeks after the operation. All trace of degenerating nerve fibres had vanished by that time; it is therefore scarcely possible that the remaining fibres of normal appearance were merely slow to degenerate. The possibility of incomplete division of the optic nerve can be dismissed, for a short length of the nerve was always removed. Thus, it may reasonably be assumed that the persisting fibres were not of retinal origin. Careful

search revealed no aberrant ganglion cells or other neurones from which the fibres might arise in the optic nerve. It is therefore concluded that these fibres constitute an efferent or centrifugal component of the nerve. The experiments showed clearly that all of them cross in the chiasma from the contralateral optic tract, and no evidence was found of an inter-retinal connexion. The precise origin of the efferent fibres remains unknown; it is possible, but by no means certain, that a few of them enter the optic tract from the suprachiasmatic and supraoptic nuclei of the hypothalamus.

Existing anatomical evidence of efferent optic nerve fibres in vertebrates is fragmentary, and to a large extent contradictory. According to Kappers, Huber & Crosby (1936) they have been reported in certain fishes, birds and mammals, but apparently not in reptiles. Many of these reports were based on the examination of normal material; as such they are of doubtful significance owing to the relatively small number of the fibres concerned, and to the virtual impossibility of determining from normal preparations the direction in which impulses are conducted. Ramon y Cajal (1911), in the course of detailed studies on the retina of birds and mammals, described the terminal arborization of certain fibres in relation to the amacrine cells. He believed these to be centrifugal optic nerve fibres, but was uncertain of their origin. Most of the experimental evidence so far advanced is also inconclusive; but the experiments of Wallenberg (1898) and of Kosaka & Hiraiwa (1915) provide convincing evidence that the tractus isthmo-opticus in birds is indeed a centrifugal pathway. In certain marsupials, on the other hand, more recent work in which silver impregnation was used (Bodian, 1937; Packer, 1941) failed to reveal any persisting optic nerve fibres after removal of an eye. It is clear that a comprehensive investigation of this problem, using modern experimental techniques and including representatives of all the vertebrate classes, will be necessary to elucidate the true state of affairs.

At present one can only speculate on the functional significance of the efferent fibres which are evidently present in *Natrix*. It is conceivable that they play some part in the control of pupillary reflexes. Alternatively, they may serve as a pathway for the control of photomechanical changes in the pigment epithelium and photoreceptors of the retina (Engelmann, 1885). The latter hypothesis gains support from physiological evidence that efferent optic nerve fibres participate in retinal photomechanical changes in *Ameiurus* (Arey, 1916). In other fishes and the frog, however, the retinal changes do not depend on the integrity of the optic nerve. Moreover, there is some doubt about the actual occurrence of photomechanical changes in reptiles (Walls, 1942). It may be, therefore, that the centrifugal fibres are involved in some other retinal mechanism as originally postulated by Ramon y Cajal; possibly their role is to modify, by facilitation or inhibition, the passage through the retina of centripetal impulses originating from the photoreceptors.

It is noteworthy that in *Lacerta* no persisting fibres could be found in the optic nerve after it had been cut (Armstrong, 1950), and this may indicate an essential difference between the snake and the lizard. However, the longest post-operative survival time for *Lacerta* was 11 weeks, and in the lizard a good deal of axonal debris still remained at that time. The debris may have obscured a small number of persisting fibres, and this point merits further investigation.

We may now consider the results of the present investigation in relation to the theory of the evolution of Ophidia from burrowing lizards (Walls, 1942). This theory is based primarily upon the existence of certain fundamental differences, anatomical, embryological and physiological, between the eyes of snakes and those of other vertebrates. One might imagine that such a radical reconstruction of the peripheral visual organ as that indicated by Walls would be reflected in the organization of the central visual apparatus. The primary visual centres in *Natrix* are on the whole less clearly differentiated than in *Lacerta* and many other reptiles; but they conform to a generalized reptilian pattern. Relatively poor differentiation is, in fact, characteristic of many of the cell masses in the forebrain and midbrain of snakes, and is not restricted to the visual centres. With regard to the visual pathways, it is true that certain differences were noted between *Natrix* and *Lacerta*. But it is evident from the foregoing discussion that fasciculation of the optic nerve, the presence of optic connexions with the nucleus posterodorsalis, and the existence of centrifugal optic nerve fibres are not exclusively ophidian characteristics. In brief, the results of this investigation provide no definite evidence in support of the theory advanced by Walls.

SUMMARY

1. Diencephalic and midbrain centres related to the optic tract system in *Natrix* have been described and compared with corresponding structures in other reptiles.

2. The brains of fifteen snakes were impregnated with silver at intervals up to 136 days following section of an optic nerve. Serial sections were examined for axonal and terminal degeneration. Degenerative changes were quite similar to those previously described in the lizard, but resorption of axonal debris occurred much more rapidly in the snake.

3. Experimental evidence was obtained for the following conclusions:

(*a*) The optic nerve consists mainly of fibres of retinal origin, but also contains a small number of efferent fibres.

(*b*) Most of the retinal fibres decussate at the optic chiasma, but a small proportion remain uncrossed.

(*c*) Crossed retinal fibres pass in the main and axillary optic tracts to terminate in the lateral geniculate nucleus, the nucleus geniculatus pretectalis, the nucleus lentiformis mesencephali, the nucleus posterodorsalis and the optic tectum; others pass in the basal optic root to the nucleus opticus tegmenti. There is no direct retino-hypothalamic connexion.

(*d*) Uncrossed retinal fibres run in the main optic tract and terminate in the rostral extremity of the lateral geniculate nucleus.

4. There is a close resemblance between the primary visual pathways of *Natrix* and those of *Lacerta*. They provide no evidence of a reconstruction of the ophidian visual system in phylogeny.

5. The problem of efferent optic nerve fibres and their functional significance has been reviewed and discussed in the light of the present experimental findings.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Longitudinal section through a normal optic nerve stained with cresyl violet. Columns of glial cells are seen in the centre of the nerve fascicles. (×270.)

Fig. 2. The normal appearance of fibres in the optic tract. Nonidez's method. (×1100.)

Fig. 3. Axonal degeneration in the right optic tract 10 days after section of the left optic nerve. Fusiform axonal swellings are present, and some fibres have ruptured between the swellings. Nonidez's method. (×1100.)

Fig. 4. Axonal disintegration in the right optic tract 13 days after section of the left optic nerve. Nonidez's method. (×1100.)

Plate 2

Fig. 5. Degenerating terminals and fibres in the lateral neuropil of the lateral geniculate nucleus 13 days after division of the opposite optic nerve. Nonidez's method. (×1600.)

Fig. 6. Terminal degeneration in the nucleus opticus tegmenti 11 days after division of the opposite optic nerve. Nonidez's method. (×1600.)

Fig. 7. An enlarged, thickened and strongly argentophil terminal bouton in the stratum fibrosum et griseum superficiale of the optic tectum. Opposite optic nerve cut 10 days previously. Nonidez's method. (×1600.)

Fig. 8. Degenerating terminals and fibres in the stratum zonale at the caudal extremity of the optic tectum. Opposite optic nerve cut 8 days previously. Nonidez's method. (×1600.)

Plate 3

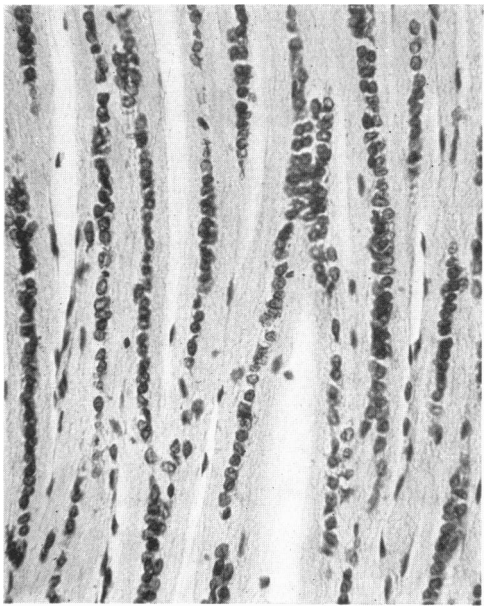
Fig. 9. Transverse section through the region of the posterior commissure 136 days after division of the left optic nerve. Extensive degeneration has occurred in the contralateral optic tectum, and is most obvious in the stratum opticum. Note also the disappearance of neuropil from the contralateral nucleus posterodorsalis. Nonidez's method. (×105.)

Fig. 10. Transverse section through the optic chiasma 136 days after section of the left optic nerve. A fascicle of uncrossed fibres is shown turning from the right (normal) optic nerve on to the ventral surface of the degenerated homolateral optic tract. Nonidez's method. (×420.)

Fig. 11. Longitudinal section through the central stump of the left optic nerve 77 days after it had been divided. Several persisting fibres of normal appearance are present; such fibres were seen throughout the nerve. Nonidez's method. (×540.)

List of Abbreviations

Dec. supraopt. vent.	Decussatio supraoptica ventralis
N. dors. med. ant.	Nucleus dorsomedialis anterior
N. gen. lat.	Nucleus geniculatus lateralis
N. gen. lat. (*lat.*)	Nucleus geniculatus lateralis (lateral neuropil)
N. gen. lat. (*med.*)	Nucleus geniculatus lateralis (medial cell plate)
N. gen. pret. (*lat.*)	Nucleus geniculatus pretectalis (lateral neuropil)
N. gen. pret. (*med.*)	Nucleus geniculatus pretectalis (medial cell plate)
N. lent. mes.	Nucleus lentiformis mesencephali
N. ovalis	Nucleus ovalis
N. paravent.	Nucleus paraventricularis
N. rot.	Nucleus rotundus
Tect. opt.	Tectum opticum
Tr. opt.	Tractus opticus



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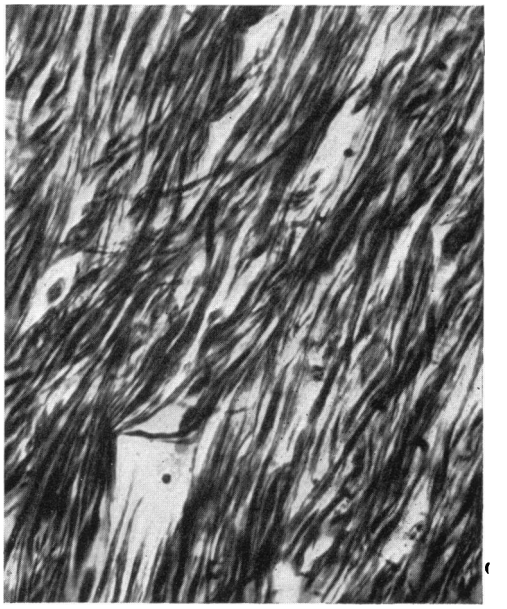

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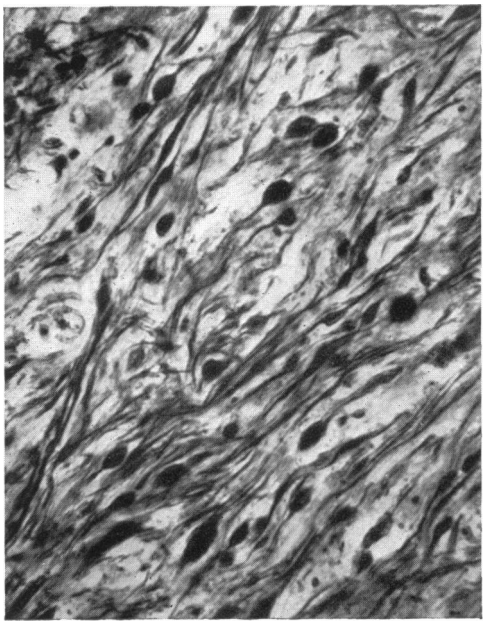

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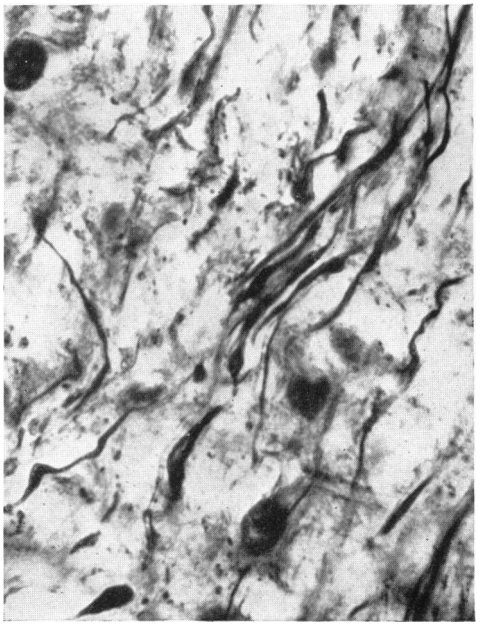
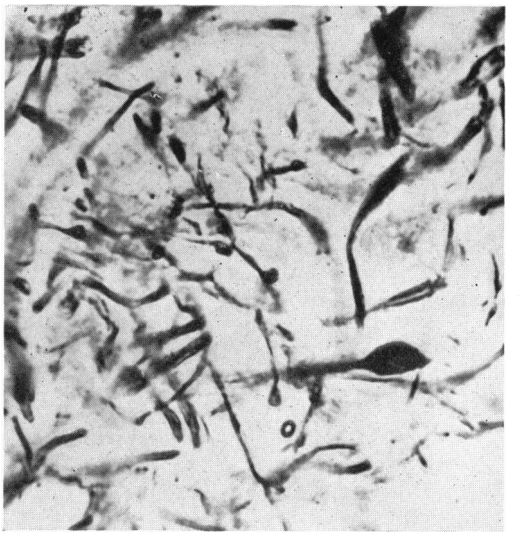


Fig. 4.

Fig. 5.



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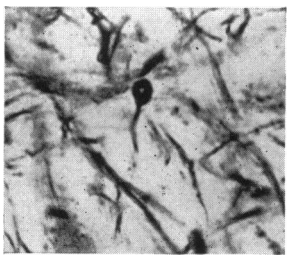

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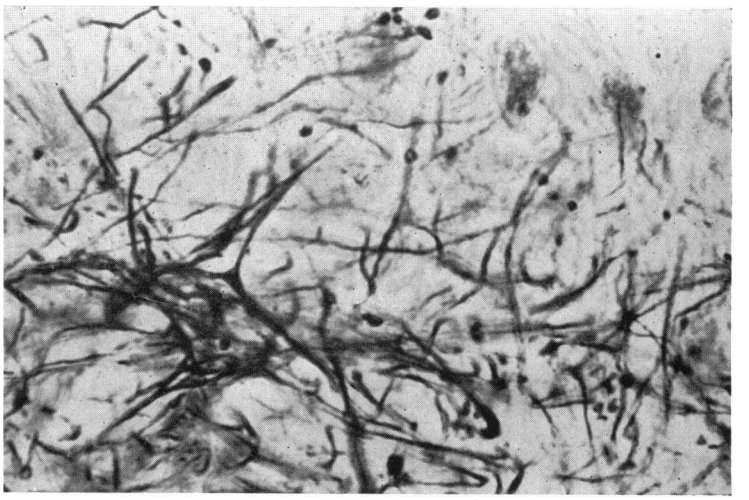

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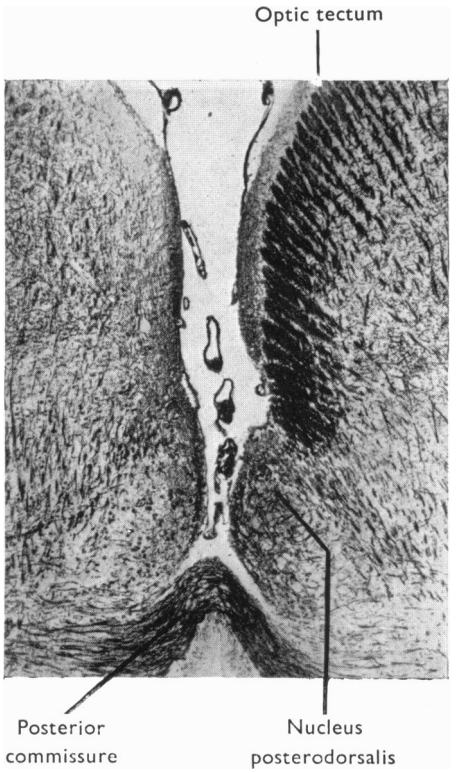

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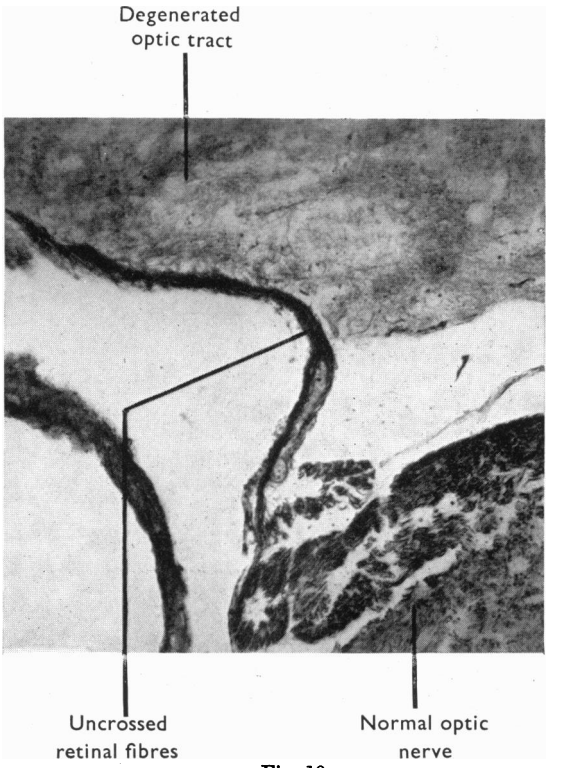

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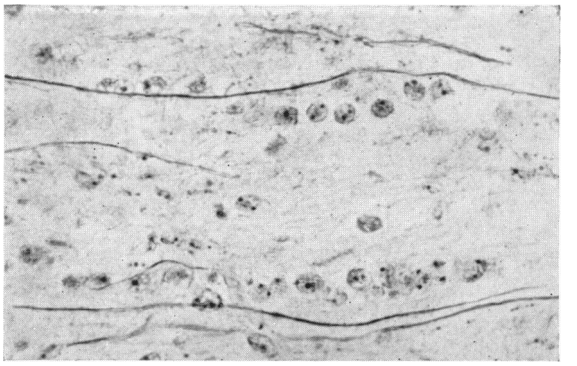

Fig. 11.